

# HISTOCHEMICAL AND IMMUNOHISTOCHEMICAL ANALYSIS OF HERNIATED DISC TISSUE REMOVED SURGICALLY FROM DOGS

G. Beha, L. Pisoni, C. Bombardi, G. Avallone, G. Sarli, S. Del Magno, F. Cinti, L. Mandrioli, G. Gandini and C. Benazzi

*Department of Veterinary Medical Sciences, University of Bologna, Italy*

**Introduction:** Herniation of intervertebral discs is a common disease in dogs that usually necessitates surgery. The aim of this study was to apply modified histological grading of herniated disc material (HDM) to surgical samples.

**Materials and Methods:** Surgically sampled HDM collected from 29 dogs processed for routine histology, histochemistry (Gruber's stain) and immunohistochemistry (Factor VIIIra). Follow-up minimum length was 3 months. Inflammation was added as a parameter in the grading system.

**Results:** All cases submitted contained nucleus pulposus, while annulus fibrosus was inconsistently available. Presence of inflammatory cells was associated with fibrosis, mineralization and neo-angiogenesis. The amount of proteoglycans (stained blue with Gruber's stain) of the nucleus pulposus decreased proportionally with the increase of chondrocyte (stained red) proliferation. Most of the samples were scored as grade 3 in a range from 1 to 5.

**Conclusions:** The variability of histological material, sampled *in vivo* and usually consisting only of the nucleus pulposus, suggests that scoring of the annulus fibrosus should be separated from that of the nucleus pulposus. The association between proteoglycan reduction and chondrocytes indicates the degree of degeneration. Finally, these data suggest that the applied histochemical staining and the modified scoring with the addition of the inflammatory parameter are appropriate to evaluate the degree of degeneration of disc material.

# AXONAL INJURY AND IMBALANCE OF GROWTH-PROMOTING AND REGENERATION-INHIBITORY AXONAL MOLECULES IN CANINE SPINAL CORD INJURY

C. Lempp\*, I. Spitzbarth\*, F. Seehusen\*, V.M. Stein†, A. Tipold† and W. Baumgärtner\*

*\*Department of Pathology and †Small Animal Clinic, University of Veterinary Medicine, Hannover, Germany*

**Introduction:** Spinal cord injury (SCI) due to intervertebral disc disease (IVDD) in dogs is regarded a suitable, spontaneously occurring animal model for human SCI research. However, mechanisms of axonal injury and impairment of axonal transport mechanisms, especially concerning processes of inhibition and promotion of axonal regeneration, respectively, are poorly understood.

**Materials and Methods:** Formalin-fixed and paraffin wax-embedded sections of healthy and injured spinal cords from dogs with acute and subacute IVDD have been investigated morphologically and immunohistochemically using antibodies against alpha-acetylated tubulin,  $\beta$ -tubulin III, dynein, kinesin and tau for quantitative evaluation of damage to structures involved in axonal transport mechanisms. Furthermore, antibodies against EPO, EPO-R, HIF, LINGO-1 and Nogo-A were used to detect axonal outgrowth and its inhibition.

**Results:** Severe changes in axonal transport mechanisms with accumulation of motor proteins, especially in swollen axons, could be detected. Significant up-regulation of LINGO, EPO, EPO-R and HIF was evident. No changes were detectable for Nogo-A.

**Conclusions:** Though canine SCI is characterized by a marked up-regulation of molecules beneficial for axonal outgrowth, a dominating impairment of axonal transport mechanisms may potentially inhibit effective axonal regeneration in SCI.

# CEREBELLAR ABIOTROPHY IN TWO RELATED LION-TAILED MACAQUES (*MACACA SILENUS*)

A.J. Malbon\*, E. Ricci\*, S. Unwin† and J. Chantrey\*

*\*Veterinary Pathology, School of Veterinary Science, University of Liverpool and †Chester Zoo, Cheshire, UK*

**Introduction:** Cerebellar abiotrophy is a degenerative condition characterized by either early or late onset of severe neurological deficits caused by the marked depletion of Purkinje cells and granule cell neurons of the cerebellar cortex. The condition has been reported in numerous species with a proposed genetic basis of transmission. Here we present the anatomopathological investigation of two closely related lion-tailed macaques. Both cases, a 9-month-old male and a 4-month-old female, shared a long history of progressively worsening ataxia, incoordination and delayed body growth.

**Materials and Methods:** Following post-mortem examinations, full sets of tissues were submitted for histopathological examination by routine haematoxylin and eosin staining. Special stains and immunohistochemistry were performed on selected serial cerebellar sections.

**Results:** Within the cerebellum, there was a moderate shrinkage of the rostromedial folia correlating with a diffuse and severe loss of Purkinje and granule cells in association with severe atrophy of the cerebellar cortex, accumulation of CSF within the subarachnoid space, reduction of myelinated fibres within the cerebellar folia and increased prominence of Bergmann glia.

**Conclusions:** Based on the characteristic findings, diagnoses of cerebellar abiotrophy were made. The relatedness of the two cases strongly supports an inherited mode of transmission. To the authors' knowledge, this is the first report of cerebellar abiotrophy in a macaque species.

# MARKED INDUCTION OF IL-8 AND IL-12 IN NEONATAL OVINE LUNG FOLLOWING EXPERIMENTAL INOCULATION OF BOVINE RESPIRATORY SYNCYTIAL VIRUS

E. Redondo, A. Gázquez, A. García, A. Franco and J. Masot

*Animal Medicine Department, Veterinary Faculty, University of Extremadura, Cáceres, Spain*

**Introduction:** This study sought to determine the immunohistochemical expression of interleukin (IL)-1 $\beta$ , tumor necrosis factor (TNF)- $\alpha$ , interferon (IFN)- $\gamma$ , IL-4, IL-6, IL-8, IL-10 and IL-12 and to measure the levels of these cytokines in lung from lambs infected experimentally with bovine respiratory syncytial virus (BRSV).

**Materials and Methods:** Lambs ( $n = 15$ ) were inoculated at 2 days of age with 20 ml of viral inoculum ( $1.26 \times 10^6$  TCID<sub>50</sub> per ml) or sterile media ( $n = 15$ ). Rectal temperature, pulse and respiratory rates were monitored daily. Lambs were subjected to necropsy examination at 1, 3, 5, 7 and 15 days post inoculation. ExtrAvidin peroxidase immunohistochemistry and ELISAs were used to detect BRSV antigen, IL-1 $\beta$ , IL-4, TNF- $\alpha$ , IFN- $\gamma$ , IL-6, IL-8, IL-10 and IL-12 in paraffin wax-embedded sections and in lung extracts, respectively.

**Results:** Inflammatory cytokines IL-8 and IL-12 were expressed in greater quantities than IL-1 $\beta$ , TNF- $\alpha$ , IFN- $\gamma$  and IL-6 in the lung of experimentally infected lambs.

**Conclusions:** These observations, together with the results of ELISA, led to consideration of important implications for therapeutic strategies based on the modulation of inflammatory cytokines in the treatment of this illness. The pharmaceutical agents that inhibit synthesis of IL-8 and IL-12 or antagonize its biological effects, could be more efficient in the treatment of BRSV infection in lambs than those that are directed only at other inflammatory cytokines.